CACHE Challenge #1 - Experimental Validation
## Preliminary Results

March 21st 2023

The Challenge

- CACHE participants were asked to use their computational methods to predict ligands binding the central cavity of the WDR domain of LRRK2. There is so far no known molecule for this binding site. See details

- After a double-blind peer review where each applicant reviewed 5 applications, 23 participants joined the challenge, representing a diverse array of physics-based and AI computational methods.

- Participants collectively selected 1955 compounds (no more than 100 compounds per participant) that we ordered and received from Enamine.

- All experimental data, the structure of the compounds and their associated computational methods will be publicly released at the end of this Challenge.
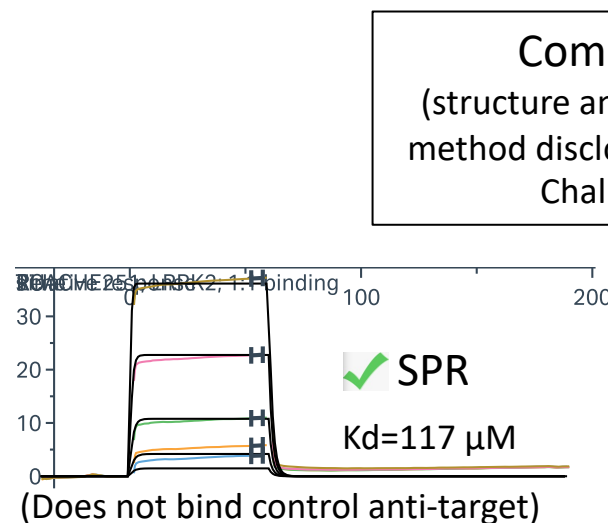
Experimental Validation

- Compounds were first screened at 50 µM and 100 µM in a surface plasmon resonance (SPR) binding assay where biotinylated LRRK2-WDR was captured on a streptavidin chip.

- >200 compounds with a binding signal between 50% and 200% of the expected value were cherry picked for dose-response experiment in the same assay

- 73 compounds from 18 participants produced a dose response and measurable K_D below 150 µM and were selected for hit expansion, where participants can select up to 50 follow-up compounds for experimental characterization.

- <u>The hit expansion round will be critical to build confidence in these 73 hit candidates</u>. We expect that progressable hits will produce a clear structure-activity relationship (SAR).
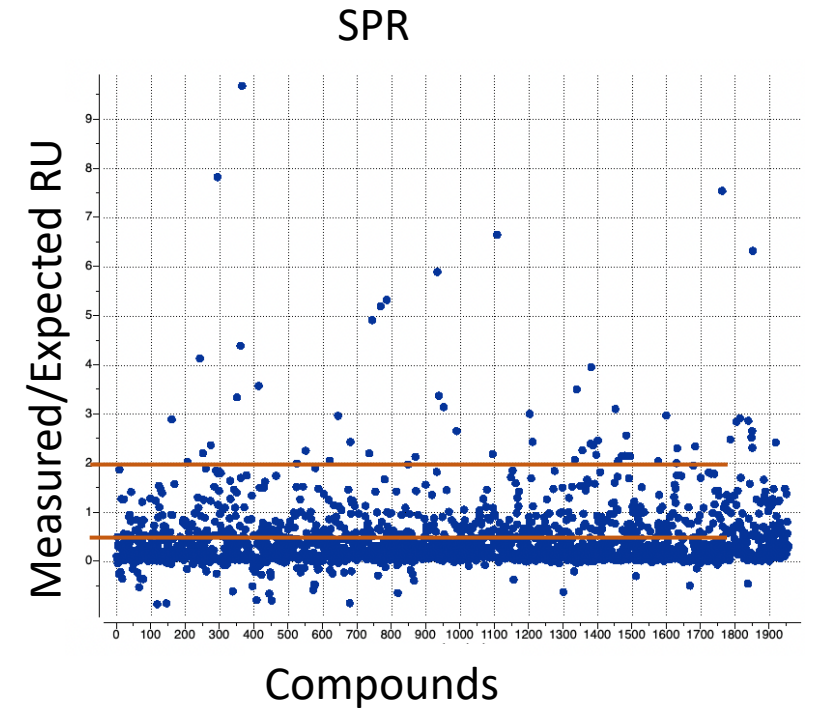
SPR

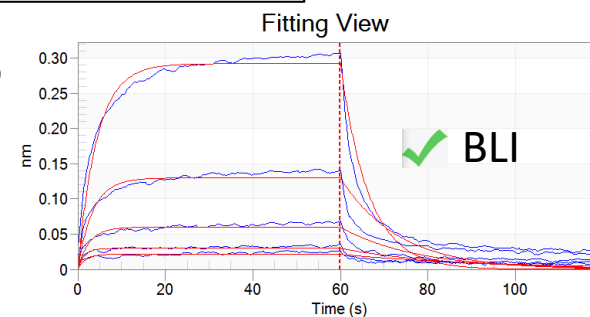Measured/Expected RU

Compounds

Some of the predicted molecules produced promising data across multiple assays. We hope the hit-expansion round will generate convincing SAR.

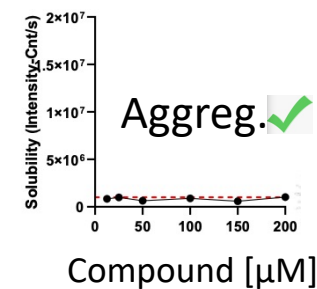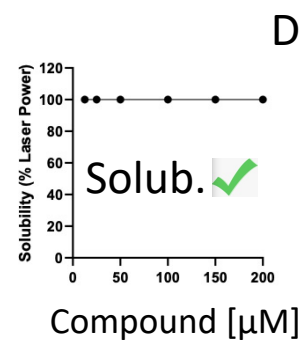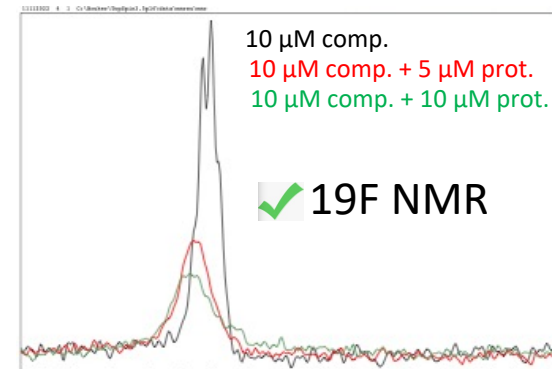Compound X
(structure and computational method disclosed at the end of Challenge #1)

Binds LRRK2-WDR in SPR assay and is specific

✅ SPR

Kd=117 μM

(Does not bind control anti-target)

Binds LRRK2-WDR in biolayer interferometry assay

Fitting View

✅ BLI

10 μM comp.
10 μM comp. + 5 μM prot.
10 μM comp. + 10 μM prot.

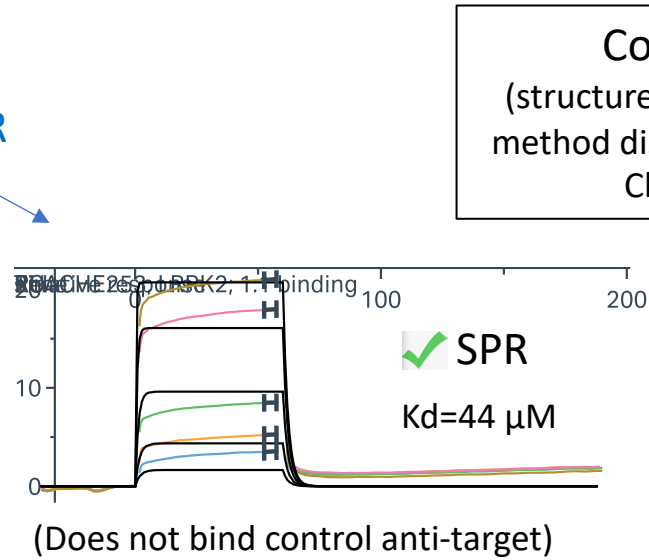✅ 19F NMR

DLS

Solub. ✅

Aggreg. ✅

Compound [μM]

Compound [μM]

Binds untagged LRRK2-WDR in 19F NMR assay

Is soluble and does not aggregate at 200μM in dynamic light scattering assay

Some of the predicted molecules produced promising data across multiple assays. We hope the hit-expansion round will generate convincing SAR.

Compound Y
(structure and computational method disclosed at the end of Challenge #1)

Binds LRRK2-WDR in SPR assay and is specific

✅ SPR

Kd=44 µM

(Does not bind control anti-target)

Binds untagged LRRK2-WDR in isothermal titration calorimetry assay

✅ ITC
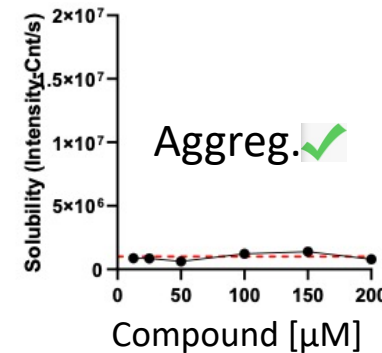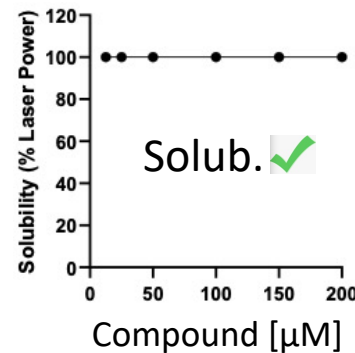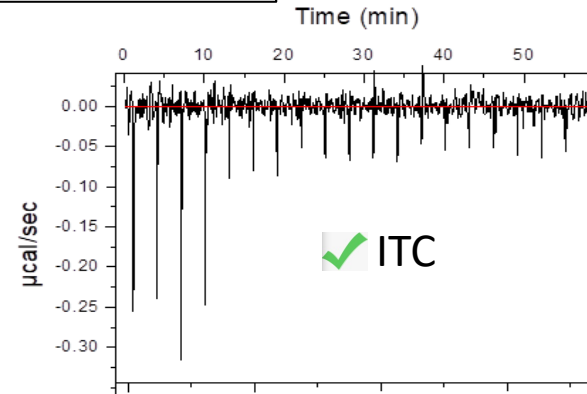
✅ Solub.

✅ Aggreg.

Is soluble and does not aggregate at 200µM in dynamic light scattering assay

ACKNOWLEDGEMENTS

CACHE #1 Experimental Platform: Structural Genomics Consortium, University of Toronto

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